REMARKS

Amendments

Claims 1 and 11 have been amended to recite that the nucleic acid encoding an interfering RNAi molecule is inserted at a BsteII site within a non-essential stem region of the adenoviral VA1 coding sequence. Support for this amendment can be found, for example, in original claims 2 and 3, in Example 1 and in Figures 1A and 1B.

Claims 2 and 3 have been canceled.

Dependent claims 17-25 have been added and recite a limitation specifying that the RNAi molecule encodes a specific nucleic acid sequence. Support for this amendment can be found, for example, in Figures 1B, 2B, paragraph 38, and under the Sequence Listing in the Specification.

Independent method claim 26 has been added. Support for this amendment can be found, for example, at paragraph 16, and Figures 1A, 2A-C, and 3.

No new matter has been added.

Summary of the Invention

In one aspect, the present invention is directed to an expression cassette which comprises an adenoviral VA1 gene and a nucleic acid encoding an interfering RNA (RNAi) molecule. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a coding sequence for the VA1 RNA. The nucleic acid is inserted at a BstEII site within a non-essential stem region of the adenoviral VA1 coding sequence as shown in Figure 2C. The nucleic acid encodes a hairpin siRNA (shRNA) or a precursor microRNA (precursor miRNA). The RNAi molecule is a substrate for Dicer (i) upon expression of the VA1 RNA which includes the RNAi molecule and (ii) after the RNAi molecule is processed out of the VA1 RNA. That is, in order to be a substrate for Dicer, the RNAi molecule must be removed from the VA1 RNA. As shown in Figure 3, the RNAi molecule is cleaved from the VA1 RNA (processed intermediate) and this processed intermediate is then a substrate for Dicer which produces the active siRNA or miRNA molecule. Hybridization of the active siRNA molecule to the mRNA in the cell leads to cleavage of the mRNA.

In a second aspect, the present invention is directed to a mammalian cell into which the above-defined expression cassette has been introduced.

Rejection Under 35 U.S.C. § 112

The Examiner rejected claims 1-3, 5, 6, and 11-16 under 35 U.S.C. § 112, first paragraph as failing to comply with the written description requirement. In particular, the Examiner argues that the limitation "corresponding to a secondary stem loop structure of the VA1 transcript" in independent claims 1 and 11 appears to be new matter unsupported by the written description. According to the Examiner, "the skilled artisan would not be able to reasonably extrapolate from the disclosure in the specification to a genus of VA1 coding sequences corresponding to a secondary stem loop structure of the VA1 transcript."

Applicants have amended claims 1 and 11 to specify that the nucleic acid encoding an interfering RNAi molecule is inserted at a BsteII site within a non-essential stem region of the adenoviral VA1 coding sequence. Applicants submit that this amendment obviates this rejection.

In view of the above amendments and remarks, Applicants submit that the claims are fully described by the specification. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner rejected claims 1-3 and 11-16 under 35 U.S.C. 103(a) as being unpatentable over Agami et al. (US 7,241,618) and Cagnon et al. (*Antisense & Nucl Acid Drug Develop* **10**:251-261, 2000) taken with Doglio et al. (US 5,837,503) in further view of either Yu et al. (*Proc Natl Acad Sci USA* **99**:6047-6052, 2002) or Ambros (*Cell* **107**:823-826, 2001).

Independent claim 1 is directed to an expression cassette comprising an adenoviral VA1 gene and a nucleic acid encoding an interfering RNA (RNAi) molecule. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a coding sequence for the VA1 RNA. The nucleic acid is inserted within the adenoviral VA1 coding sequence. The nucleic acid encoding the RNAi molecule encodes a hairpin siRNA (shRNA) or a precursory microRNA (precursor miRNA). Upon

expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer.

Independent claim 11, from which claims 12 and 15-16 depend, is directed to a mammalian cell into which the above expression cassette has been introduced.

The claimed RNAi molecule is a substrate for Dicer (i) upon expression of the VA1 RNA which includes the RNAi molecule and (ii) after the RNAi molecule is processed out of the VA1 RNA. Thus, in order to be a substrate for Dicer, the RNAi molecule must be removed from the VA1 RNA. It is clear from Figure 3 that the RNA molecule is cleaved from the VA1 RNA as a processed intermediate and this processed intermediate is then a substrate for Dicer which produces the active siRNA or miRNA molecule. Thus, the specification teaches and the claims require that the RNAi molecule must be processed from the VA1 RNA before it can become a substrate for Dicer.

The Examiner relies on Agami et al.'s disclosure of making and using an expression cassette comprising an adenoviral VA1 promoter operably linked to an siRNA molecule, wherein the siRNA molecule can be shRNA (col. 50-51; Figures 8 and 10; claim 3). The Examiner primarily relies on Cagnon et al. and Doglio et al. for disclosures of the structural limitations of the claimed product. The Examiner relies on Cagnon et al.'s disclosure of inserting an RNAi molecule into a VA1 expression cassette using a filled-in Not1 site that was ligated into the BstEII cleaved, filled in vector (page 252). Compare Figure 3A of Cagnon et al. with Figure 1A of the application. The Examiner relies on Doglio et al.'s disclosure of inserting an expression cassette (comprising an oligonucleotide) between or outside the boxes A and B constituting the promoter of the VA gene or into VA1 gene (col. 3, lines 5-10). Based on these disclosures in Cagnon et al. and Doglio et al., the Examiner concludes that the structural limitations of the claimed product were known in the art for delivering a nucleic acid inhibitor to the cytoplasm of a cell. The Examiner also relies on Yu et al. for teaching an RNA polymerase III vector comprising shRNA can inhibit expression in mammalian cells, and Ambros for teaching microRNA.

The Examiner admits that Cagnon et al., Doglio et al., and Rossi et al. (US 6,100,087) teach that inhibitory RNA that is inserted in a VA1 transcript is not cleaved from the VA1 transcript, and that the prior art is "silent with respect to the limitations 'wherein upon expression the VA1 RNA

contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer.'" See Office Action, pages 7 and 8. However, the Examiner argues that the prior art "makes obvious all of the structural limitations of the claimed product and the motivation to produce and a reasonable expectation to make said product, so the functional effect of the claimed product are considered to be inherent of the product."

In response to Applicants' prior argument that there is (no reasonable expectation of success and thus) no motivation to combine in view of the teachings in Cagnon et al., Doglio et al., and Rossi et al. that inhibitory RNA that had been inserted in a VA1 transcript is not cleaved from the VA1 transcript, the Examiner argues that one of ordinary skill in the art would have been motivated to use the construct taught by Cagnon et al. since the construct results in cytoplasmic transport of the RNAi molecule and Dicer is located in the cytoplasm. According to the Examiner, it would have been obvious to try for co-localization of the VA1 transcript with dicer and cytoplasmic RNAi machinery.

Applicants traverse the rejection.

Applicants submit that Agami et al.'s disclosure of the use of the VA1 promoter to drive expression of a desired sequence is simply a disclosure of potential RNA poly III promoters that can be used for the expression of a desired sequence. There is no disclosure in Agami et al. as to how the VA1 promoter would be used to make a construct for expressing a desired sequence. Applicants further submit that it was well known in the art at the time that Agami et al. was filed, that the VA1 promoter was used as a conventional promoter by simply using the promoter elements to drive expression of the desired sequence and not to express the desired sequence as part of the VA1 RNA transcript. For example, see Shen et al. (*Intl Immunology* **13**:665-674, 2001; copy submitted herewith), which teaches using only the promoter elements. Shen et al. discloses that the VA1 promoter was used in the conventional manner, i.e., as a promoter for expression of a nucleic acid in which the nucleic acid to be expressed was positioned so that it would be expressed separately and not as part of a VA1 RNA.

However, the claims require that "upon expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer." One skilled in

the art would not look to Agami et al. in order to express the VA1 gene and the RNAi molecule. Furthermore, there is no disclosure or suggestion in Agami et al. to use the VA1 promoter elements in the manner proposed by the Examiner.

The Examiner cites Cagnon et al. and Doglio et al. for showing how the VA1 promoter could be used, i.e., by insertion of a desired sequence, such as an inhibitory RNA, into the coding sequence of the VA1 gene such that the transcribed VA1 RNA includes the desired sequence. The inhibitory RNA of Cagnon et al. is either an antisense or a ribozyme. The inhibitory RNA of Doglio et al. is an antisense. In addition to Cagnon et al. and Doglio et al., Applicants also note that Rossi et al. (US 6,100,087) contains a similar disclosure in which the inhibitory RNA is a ribozyme. All three of these references teaches that the inhibitory RNA that is inserted into the VA1 RNA coding sequence and expressed as part of the VA1 RNA is not processed, e.g., cleaved from the resultant VA1 RNA. Instead, Cagnon et al., Doglio et al. and Rossi et al. teach that the inhibitory RNA, e.g., antisense or ribozyme, are active within the resultant VA1 RNA. Thus, the VA1 RNA containing the inhibitory RNA of Cagnon et al., Doglio et al. and Rossi et al. is not processed from the VA1 RNA and thus does not become a substrate for Dicer.

In view of the teachings in Cagnon et al., Doglio et al., and Rossi et al., Applicants submit that these references teach away from using the VA1 gene in expression construct containing an shRNA or miRNA inhibitory RNA altogether because these references teach that the inhibitory RNA remains a part of the VA1 RNA and is not processed or cleaved from the VA1 RNA. Applicants also submit that it was well known in the art that shRNA or precursor miRNA had to be processed by Dicer in order to become a functional molecule. Thus, Applicants submit that the skilled artisan would have been motivated to utilize a promoter system which would produce a shRNA or precursor miRNA that would have been expected to be accessible in order to be a substrate for Dicer. Because the prior art cited by the Examiner, specifically, Cagnon et al. and Doglio et al., as well as Rossi et al. cited by Applicants, teach that the inhibitory RNA inserted into the VA1 gene is not cleaved from the VA1 RNA but is active. Since these inhibitory RNAs are not cleaved from the VA1 RNA, Applicants submit that the skilled artisan would not have been motivated to use the VA1 promoter in the manner taught by Cagnon et al. and Doglio et al., but

would have been motivated to use the VA1 promoter as a conventional promoter as taught by the prior art, such as Shen et al. (copy submitted herewith). Applicants submit that there is no teaching or suggestion in any of the prior art cited by the Examiner that would lead a skilled artisan to any reasonable expectation that a shRNA or precursor miRNA would be processed from the VA1 RNA transcript. Since there is no such reasonable expectation in the art, Applicants submit that there is no motivation to make the construct proposed by the Examiner, especially in view of specific teachings in the art teaching away from such a construct.

Furthermore, Applicants submit that the prior art does not teach or suggest the claimed construct, and thus does not provide a construct with all of the structural limitations of the claimed construct because (i) the prior art contains no motivation to insert a shRNA or precursor miRNA into a VA1 coding sequence and (ii) the prior art specifically teaches away from making such an insertion. There is no motivation because the cited prior art teaches that the inserted inhibitory RNA is not cleaved from the VA1 RNA transcript. Thus, Applicants submit that the prior art specifically teaches away from the motivation contended by the Examiner. In addition, Applicants submit that understanding conventional use of a VA1 promoter, such as shown by Shen et al. (copy submitted herewith), and in view of the specific teachings of Cagnon et al., Doglio et al., and Rossi et al., one skilled in the art would not have been motivated to make the construct proposed by the Examiner. Accordingly, Applicants submit that the Examiner has not cited prior art that would have the claimed structural limitations. Consequently, Applicants submit that the combination of Agami et al., Doglio et al, and either Yu et al. or Ambros do not render the claimed subject matter obvious.

In view of the above amendments and remarks, Applicants submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al, and either Yu et al. or Ambros. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner rejected claims 1, 5, and 6 as being unpatentable over either Agami et al. and Cagnon et al. taken with Doglio et al. and either Yu et al. or Ambros, as applied to claims 1-3 and 11-16 above, and further in view of Lorens (US 2004/0005593).

Dependent claim 5 adds the limitation to claim 1 that the RNAi molecule encoding nucleic acid comprises a loop containing from 4 to 9 bases. Dependent claim 6 further adds the limitation that the loop contains 8 bases.

The Examiner relies on the same disclosures in Agami et al., Cagnon et al., Doglio et al., Yu et al., and Ambros as set forth in the rejection of claims 1-3 and 11-16 above. The Examiner relies on Lorens' disclosure of an RNAi molecule having a loop containing at least 6 nucleotide bases (p. 7).

According to the Examiner, it would have been *prima facie* obvious to one skilled in the art to combine the prior art references to produce an expression cassette comprising an adenoviral VA promoter, wherein an RNAi molecule comprises a loop containing about 8 nucleotide bases. One skilled in the art would have been motivated to combine the teaching to determine if there is an increase in the inhibition by using a common structure in a shRNA or precursor miRNA molecule to make the expression cassette.

Applicants traverse this rejection for the same reasons discussed above. Further, the new combination of references does not remedy all deficiencies or refute the arguments above. Thus, Applicants submit that either Agami et al. and Cagnon et al. taken with Doglio et al. and either Yu et al. or Ambros, and further in view of Lorens does not render the claimed subject matter obvious.

In view of the above amendment and remarks, Applicant submit that the claimed subject matter is not rendered obvious by the combination of Agami et al. or Cagnon et al. and Doglio et al., and Yu et al. or Ambros, and Lorens. Withdrawal of this rejection is requested.

Patentability of Newly Added Claims

Claims 17-25 are distinguishable from the prior art because the specific sequences are not disclosed in the references.

Claim 26 is distinguishable from the prior art based on step limitations specifying that the RNAi molecule is inactive within the VA1 RNA and that only the cleaved RNAi molecule is a substrate for Dicer. In particular, Cagnon et al., Doglio et al., and Rossi et al. each fail to explicitly disclose an inactive RNAi molecule within the VA1 RNA and a processed intermediate.

Conclusion

In view of the above amendments and remarks, Applicants believe that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application.

Respectfully submitted,

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Enclosure: Shen, H.M. et al. The 3' Igκ enhancer contains RNA polymerase II promoters: implications for endogenous and transgenic κ gene expression. *Intl Immunology* **13**:665-674 (2001).